

## Unit 3: The Microenvironment, Its Role in Cell Fate Decisions and Cancer

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### California State Standards

#### Biology/Life Science

- 1.a. Students know cells are enclosed within semi-permeable membranes that regulate their interaction with their surroundings.
4. Genes are a set of instructions encoded in the DNA sequence of each organism that specify the sequence of amino acids in proteins characteristic of that organism.
- 4a. Students know the general pathway by which ribosomes synthesize proteins, using tRNAs to translate genetic information in mRNA. *"After learning about transcription and translation through careful study of expository texts, students can simulate the processes on paper or with representative models."*
- 4b. Students know how to apply the genetic coding rules to predict the sequence of amino acids from a sequence of codons in RNA. *"In eukaryotes, the initial RNA transcript, while in the nucleus, is composed of exons, sequences of nucleotides that carry useful information for protein synthesis, and introns, sequences that do not. Before leaving the nucleus, the initial transcript is processed to remove introns and splice exons together. The processed transcript, then properly called mRNA and carrying the appropriate codon sequence for a protein, is transported from the nucleus to the ribosome for translation."*
- 4d. Students know specialization of cells in multicellular organisms is usually due to different patterns of gene expression rather than to differences of the genes themselves. *"Nearly all cells in an organism contain the same DNA, but each cell transcribes only that portion of DNA containing the genetic information for proteins required at that specific time by that specific cell. The remainder of the DNA is not expressed. Specific types of cells may produce specific proteins unique to that type of cell."*
- 5.b. Students know how to apply base-pairing rules to explain precise copying of DNA during semiconservative replication and transcription of information from DNA into mRNA.

### Goals

- Understand that genes are a set of instructions, encoded by the DNA sequence, that denote the types and sequence of amino acids during protein synthesis.
- Demonstrate the central dogma of biology by modeling the transcription of a DNA sequence to mRNA and the translation of mRNA codon triplets into amino acids that fold to form a protein.
- Explain the significance of signals from the microenvironment and gene expression in the formation of specialized cells in multicellular organisms.

### Objectives

1. Students will be able to define genotype, phenotype, and microenvironment in terms of "cell fate decisions" and how a cell is able to specialize appropriately.
2. Students will be able to differentiate between genotype and gene expression in terms of protein synthesis (which results in a phenotype).
3. Students will be able to explain the relationship between DNA, mRNA, and tRNA as well as the processes used to form a protein during protein synthesis.
4. Students will be able to simulate the production of different types of mature cells from an adult stem cell depending on signals from the microenvironment using the framework of the central dogma of biology. AP students can begin to understand the limitations of the central dogma, in that RNA often regulates gene expression.
5. Students will be able to recognize factors in the microenvironment that control gene expression and cell fate, including signaling proteins, extracellular matrix proteins, forces, and cell-to-cell interactions.
6. Students will be able to describe how factors in the microenvironment signal stem cells to proliferate or differentiate (for example, a mammary gland progenitor cell).
7. Students will be able to investigate applications of appropriate cell responses to signaling in colon stem cells and hair follicles while exploring how aberrant gene expression could but does not always lead to cancer.
8. Students will be able to simulate the role of the microenvironment by understanding array systems and by modeling them during the Microenvironment Array activity.

## UNIT OUTLINE

### I. Invitation

#### A. Show animation: Colony Stimulating Factor

Scroll to bottom to find CSF animation.

##### SYNOPSIS:

When the immune system recognizes a pathogenic invasion, immune cells respond by releasing colony stimulating factors (CSF), which stimulate stem cells in the bone marrow to construct specialized white blood cells. These white blood cells begin to form a colony, which will continue to undergo cell division. As the colony grows and matures, the formation of an immune cell "army" then travels through the circulatory system with a mission to eradicate the infection. Since its discovery, CSF has helped millions of cancer patients survive destruction to the bone marrow that often results from high-dose chemotherapy.

#### B. For an introduction or review of the genetic processes underlying the above proliferation, show these animations on transcription and translation and ask students why these processes are important to the effects of CSF on bone marrow stem cells.

1. Watch the following animations:
  - a. DNA Central Dogma Part 1 - Transcription
  - b. DNA Central Dogma Part 2 - Translation
2. Have students practice transcription and translation using this interactive animation.

#### C. AP Extension activity: How does a cell with one genotype give rise to cells with different phenotypes?

1. Choices of a Common Myeloid Progenitor cell
  - a. Become erythrocytes (red blood cells)
  - b. Become granulocytes (white blood cells)
2. "From Genotype to Phenotypes: Red Blood Cell or White Blood Cell?" activity. This activity requires preparation in advance.  
Appendix A: From Genotype to Phenotypes  
Appendix A: Instructions for From Genotype to Phenotypes activity
3. In this exercise, your students will simulate the production of EITHER red blood cells OR white blood cells from common myeloid progenitor cells. After receiving instruction from the teacher to use one of these signaling molecules,
  - a) Erythropoietin OR b) Granulocyte-Colony Stimulating Factor, students will go through the process of transcription and translation to create either a) RED protein or b) WHITE protein.
4. Download Another Plasmid Editor (ApE) for teacher and all student computers; required for this activity.

### II. Exploration

#### A. Cell fate and behavior

1. Lecture and Discussion: How does cell phenotype come from genotype?
  - a. We have 30,000 genes, but only a subset are expressed in each cell.
  - b. Through gene expression, transcription and translation of this subset of genes determines how a cell looks and acts (its phenotype).
  - i. Phenotype includes but it not limited to: Proteins and enzymes, surface markers, cell behaviors (migration), cell fate decisions (proliferation and differentiation).
  - c. What dictates potential? Pluripotent and multipotent stem cells respond differently to signals because they express different genes. These characteristics are result of the differential expression of subsets of genes.
  - d. How does a cell know what to become? In a cell fate decision, a stem cell makes a decision to behave, for example, proliferate, differentiate, or migrate. Is this decision random? If not, what influences this decision? (The decisions to proliferate/differentiate don't have to be mutually exclusive. For example, Granulocyte-Colony Stimulating Factor influences proliferation and then differentiation.)
  - e. Central Dogma of Biology worksheets and signaling factors

Students can read about examples of microenvironmental factors and regulation below in section 2.

Appendix B: Central Dogma student worksheet and teacher version
2. Jigsaw activity: Read the general instructions.  
Pick several of these readings for students to read and understand the effects of each microenvironmental component. Choose

one or more from sections a. through d.

Ask students to consider: What controls gene expression and cell fate? The microenvironment, which consists of: signaling factors (soluble like Colony Stimulating Factor and steroid hormones), extracellular matrix proteins, forces, and cell-cell interactions.

a. Signaling factors

EASY: Protein receptor for neural cells

EASY: Nerve growth factor

MID: Stat3 turns off immune system and allows cancer to form

MID: Protein signaling neural formation and Notch pathways

CHALLENGING: Proteins that cause stem cells to form skin cells

b. Extracellular matrix proteins

EASY: ECM protein that promotes muscle cell health

MID: ECM determines cell fate and cancer development

MID: Cell movement regulated between cells

MID: UCSD research summary of ECM role on cells

CHALLENGING: General overview on role of ECM

CHALLENGING: Role of ECM in cell differentiation

c. Forces

EASY: Dr. Sanjay Kumar press release

MID: Effects of mechanical forces on embryonic stem cells

MID: Cell orientation determines its division

d. Cell-cell interactions

MID: Hormone receptors inside cells

CHALLENGING: Inter-cell communication

CHALLENGING: Cell-cell interaction networks regulate cell fate

3. Wet lab exercise

The RNAi and Genetics lab protocol from the Sciencebridge program @ UC San Diego is an excellent way to examine the central dogma. In this lab, you will look at the microscopic nematode, *C. elegans*. These worms eat bacteria and you feed them bacteria that have been transformed with RNAi. This molecule degrades the production of a muscle protein in the worms. Over three days, the worm loses its shape and becomes "uncoordinated." You can ask students: how did changing gene expression affect the microenvironment? What are the observable changes on a macro scale? What might happen to cells as a result of this drastically changed microenvironment?

4. AP extension video and discussion: Cell invasions video

Cell behavior is similarly determined by signals from outside the cell. An example is cell migration, a property of stem cells as well as somatic cells such as fibroblasts. What determines the movement of a cell?

## B. Microenvironment and Cancer

Cells live in/on tissue (connective tissue, including other cells). The microenvironment around the cell is anything to which the cell could potentially respond by exhibiting a behavior. It also may keep cancer cells "in check."

1. Lecture using supplementary Microenvironment PowerPoint slides 1-7 and information below.

a. Two examples of normal microenvironments:

i. Intestinal Crypt Stem Cells - A Clonal Conveyor Belt animation from Molecular Movies. Scroll down to the Clonal Conveyor Belt animation. Preload, as this will take time to load. This resource contains ~100 other biological animations!

ii. Hair follicles - Examination and Web Exploration

Examine the cells in the hair follicle using pictures from this site: Integumentary System. Teacher can also use prepared slides of hair follicles. (Buy slides from Wards, Fisher, etc.)

b. Optional: Teach the different types of epithelial tissues and connective tissues using virtual labs. Guide to the Tissues

c. Discuss: What is the hair follicle microenvironment? Where are the stem cells housed and what are their microenvironments (what cells are next to them, etc.)? What might be the result of changing this particular microenvironment? Ex. Hormones (testosterone, other androgens) in or near the hair follicle increases growth of hair. Can you think of any products that increase hair growth and how they might work? (Students could search for this information online.)

2. Aberrant gene expression combined with abnormal microenvironment = Cancer: Lecture and discussion

a. Cancer is caused by mutations in proto-oncogenes, resulting in oncogene expression (lecture from Teacher Background Information)

b. There are different hypothesis to explain how cancer grows and spreads: examples are the Cancer Stem Cell hypothesis and the Clonal Evolution Model. Describe how the Cancer Stem Cell hypothesis differs from the Clonal Evolution Model. Refer to the

Figure 1 from this free article.

3. Readings: First, read the following article.

EASY-MID: Oncogenes and Tumor Suppressor Genes

Next, assign one or more of the following articles to students.

EASY/MID: Researchers Find Prostate Cancer Stem Cell

MID: The Stem Cell Hypothesis

MID: UCSD researchers pave the way for effective liver treatments

MID: Diabetes Drug Kills Cancer Stem Cells In Combination Treatment in Mice

MID: Melanoma Spawns Tumors with Deadly Efficiency

MID/CHALLENGING: Stem Cells May Be Key to Cancer

4. AP extension discussion question:

Odd gene expression patterns cause cancer, but sometimes cells with these genetic mutations are not necessarily "cancerous," because they lack the phenotype of malignant cancer cells. What could be preventing them from acting like cancer cells?

### III. Application

**A. Lecture: Human Mammary Gland Progenitor Cells, understanding the role of the microenvironment in normal development and cancer (start with slides 8-16 of the Microenvironment PowerPoint and add information about breast cancer from the Teacher Background Information.)**

**B. Inspection and understanding of arrays in general by looking at cDNA array methods and data or other types of simpler array systems like Enzyme-linked Immunosorbent Assay (ELISA).**

1. Dolan DNA Learning Center: cDNA array animation OR Microarray flash animation

2. Microbiology, Prescott, Harley, and Klein: ELISA animation

**C. Understand immunocytochemistry using fluorescently-tagged antibodies.**

Antibodies are an important research tool in cell biology. Scientists use antibodies generated in different animals to identify specific types of stem cells based on the different molecules those cells produce on their surfaces or inside their nuclei. To visualize a certain type of stem cell using fluorescence microscopy, a scientist would prepare a thin slice of tissue or a small dish of cells, then incubate the tissue or cells in what's called a "primary antibody" specific to one molecule on the surface of the stem cell of interest. This primary antibody recognizes only those molecules and attaches to them. Next, the scientist would incubate the tissue slice or cells in a "secondary antibody" fused to a fluorescent molecule. This secondary antibody only recognizes and binds to the primary antibody. Now, the scientist looks at it under a fluorescent microscope. The microscope uses a certain wavelength of light that excites the fluorescent molecules marking the stem cells of interest, causing them to emit another wavelength of light that can be detected by the microscope and your eye.

EASY: Immunofluorescence Labeling method

EASY: Fluorescently-tagged stem cell pictures on CIRM's flickr page

MID/CHALLENGING: Mammary gland picture from LaBarge lab, Lawrence Berkeley National Labs

Appendix C: Immunofluorescent labeling of mammary gland

CHALLENGING: Immunofluorescence Labeling of Cells, Sigma-Aldrich

**D. Visualize "Breast Stem Cells," "Control of Breast Stem Cells," and "Origin of Breast Cancer" in that order from Molecular movies.**

**E. Understand and summarize either orally or with a short paper the Microenvironment Array (MEArray).**

1. Mammary progenitor cell: turns into myoepithelial cells or luminal epithelial cells, but what are the signals telling them to change?

a. Lawrence Berkeley National Labs News Story: It Takes a Village

b. AP biology extension: Human mammary progenitor cell fate decisions are products of interactions with combinatorial microenvironments. Read the following abstract:

In adult tissues, multi-potent progenitor cells are some of the most primitive members of the developmental hierarchies that maintain homeostasis. That progenitors and their more mature progeny share identical genomes, suggests that fate decisions are directed by interactions with extrinsic soluble factors, ECM, and other cells, as well as physical properties of the ECM. To understand regulation of fate decisions, therefore, would require a means of understanding carefully choreographed combinatorial interactions. Here we used microenvironment protein microarrays to functionally identify combinations of cell-extrinsic mammary gland proteins and ECM molecules that imposed specific cell fates on bipotent human mammary progenitor cells. Micropatterned cell culture surfaces were fabricated to distinguish between the instructive effects of cell-cell versus cell-

ECM interactions, as well as constellations of signaling molecules; and these were used in conjunction with physiologically relevant 3 dimensional human breast cultures. Both immortalized and primary human breast progenitors were analyzed. We report on the functional ability of those proteins of the mammary gland that maintain quiescence, maintain the progenitor state, and guide progenitor differentiation towards myoepithelial and luminal lineages.

**F. Design your own MEArray research project: Help your students describe how a scientist would test putative ME factors' effects on mammary gland progenitors using MEArray technology. See MEArray Teacher Guide describing this project:**  
**Appendix D: MEArray Activity**

**G. AP Extension questions: How do forces affect cell fate decisions? Can an ME Array test this? (No, it can only test ECM molecule or signaling protein interactions and cell-cell contact, not forces.) How would you test the effects of forces on stem or progenitor cells?**

1. Cell fate determination research covers three areas:
  - a. Adhesion-dependent cell survival
  - b. Biomechanical forces and the control of cell phenotype
  - c. Adhesion-dependent manipulation of stem cell fate
2. For a MID/CHALLENGING overview of these areas, see work of Sanjay Kumar's lab, UC Berkeley Bioengineering
3. Articles about how forces affect cell fate:  
MID: Cellular Connections  
CHALLENGING: Macromolecular biophysics of the cytoskeleton  
CHALLENGING: Nonspecific colloidal forces in biomacromolecular systems  
VERY CHALLENGING: Biophysics and dynamics of natural and engineered stem cell microenvironments

#### IV. Assessment

1. Describe how a cell's genotype is converted into its phenotype.
2. What are the four components of the microenvironment?
3. What is cell behavior? Give three examples.
4. Give a specific example of how the microenvironment guides a cell fate decision.
5. Put the following terms in their proper sequence of appearance during gene expression: protein, splicing, mRNA, DNA, translation, pre-mRNA, transcription, tRNA.
6. Define a stem cell niche and give one example. (Intestinal crypt or hair follicle)
7. Describe in general how a Microenvironment Array/cDNA array/ELISA works and why a scientist would want to use it.

AP thought questions (possible web research project):

1. Is a genetic mutation necessary for cancer?
2. Could cancer occur only by manipulating the microenvironment?
3. If you took a piece of normal tissue and inserted it inside a tumor, what would happen?
4. If you infect a chicken embryo with a cancer-causing virus and the chicken grew up cancer free, could you conclude the chicken's cells were noncancerous?
5. How might we test to see if certain microenvironments can stop cancer from growing?

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**Source URL:** <https://www.cirm.ca.gov/our-progress/unit-3-microenvironment-its-role-cell-fate-decisions-and-cancer>